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(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11) EP 0 672 192 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
13.10.1999 Bulletin 1999/41

(51) Int. Cl.⁶: **A61K 35/76, C12N 7/04,
C12N 7/06, G01N 33/50,
C12Q 1/70**

(21) Application number: **93914329.3**

(86) International application number:
PCT/US93/05247

(22) Date of filing: **01.06.1993**

(87) International publication number:
WO 93/24660 (09.12.1993 Gazette 1993/29)

(54) METHOD FOR INHIBITION OF VIRAL MORPHOGENESIS

VERFAHREN ZUR HEMMUNG DER VIRALEN MORPHOGENESE

PROCEDE D'INHIBITION DE LA MORPHOGENESE VIRALE

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL
PT SE**

(30) Priority: **29.05.1992 US 890754**

(43) Date of publication of application:
20.09.1995 Bulletin 1995/38

(73) Proprietor: **Glenn, Jeffrey S.
Palo Alto, CA 94304 (US)**

(72) Inventor: **Glenn, Jeffrey S.
Palo Alto, CA 94304 (US)**

(74) Representative:
**Goldin, Douglas Michael
J.A. KEMP & CO.
14 South Square
Gray's Inn
London WC1R 5LX (GB)**

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description**Technical Field**

- 5 [0001] The invention is directed to inhibiting viral morphogenesis and viral infection. In particular, it concerns effecting such inhibition by inhibiting the prenylation or post prenylation reactions of a viral protein.

Background Art

- 10 [0002] It has been shown that certain membrane-associated proteins require the addition of lipophilic residues in order to function properly. One family of such modifications is termed "prenylation" because the hydrophobic residue is derived from isoprenoid precursors. The prenyl residue is known to attach to the sulphydryl group of a cysteine which has been shown in a number of membrane-associated proteins to be contained in a "CXXX" (SEQ ID NO:1) box at the carboxy terminus of the substrate protein. In particular, one such membrane-associated protein has been shown to be

15 the protein product of the ras oncogene. Summaries of these reactions conferring hydrophobic properties on membrane proteins, including prenylation, have appeared by Hoffman, M., *Science* (1991) 254:650-651, and by Gibbs, J.B., *Cell* (1991) 65:1-4.

[0003] In addition, in many cases, prenylation is a first step in a series of further reactions which modify the carboxy terminus of prenylated proteins. These prenylation initiated, or post-prenylation reactions include carboxymethylation and proteolysis.

[0004] In the prenylation substrate proteins studied to date, the CXXX (SEQ ID NO: 1) box contains aliphatic residues in the second and third positions and a leucine, serine, methionine, cysteine or alanine in the terminal position. Thus, in the CXXX (SEQ ID NO: 1) boxes so far studied, the box itself is relatively hydrophobic.

[0005] It has now been found that prenylation of a viral protein is necessary for the morphogenesis of hepatitis delta virus (HDV). This is the first demonstration that viral proteins are subject to prenylation. Furthermore, certain functional consequences can be ascribed to prenylation. The viral protein which is the target of prenylation, surprisingly, contains a hydrophilic CXXX (SEQ ID NO:1) box of the sequence Cys-Arg-Pro-Gln (SEQ ID NO:2). Prenylation, or prenylation-initiated modification, of this relatively hydrophilic CXXX (SEQ ID NO:1) box and corresponding CXXX (SEQ ID NO:1) boxes (hydrophilic or otherwise) or other cysteine-containing sequences near the C-terminus of proteins in other virions are suitable targets for antiviral strategies.

[0006] These targets can now be seen to include, but are not limited to, proteins of hepatitis A virus (HAV), hepatitis C virus (HCV), herpes simplex virus (HSV), cytomegalovirus (CMV), varicella-zoster virus (VZV), influenza virus, plant viruses such as tobacco mosaic satellite virus (TMSV) and barley stripe mosaic virus (BSMV), the core antigen of hepatitis B virus (HBV) and the nef gene product of human immunodeficiency virus-1 (HIV-1) -- especially since nef has been shown to play an important role in the development of AIDS. (Kesstler, H.W. III, et al. *Cell* (1991) 65:651-662. Accordingly, inhibition of the prenylation of these target proteins or the post-prenylation reactions thereof is claimed to be inhibitory to the progress of these infections.

Disclosure of the Invention

40 [0007] The invention provides methods to interfere with viral morphogenesis, production, release or uncoating both *in vitro* and *in vivo*. Agents which interfere with the prenylation of, or the post-prenylation reactions of, at least one viral protein are provided to infected cells to halt the viral infection. Such cells may be in culture or may be contained in an animal or plant subject.

45 [0008] Thus, in one aspect, the invention is directed to the use of an agent effectively interfering with the prenylation of, or the post-prenylation reactions of, at least one viral protein in the manufacture of a medicament for the inhibition of virion morphogenesis, production, release or uncoating. In another aspect, the invention is directed to an assay method for screening candidate drugs for their ability to inhibit prenylation. In a third aspect, the invention is directed to the use of an agent effective to inhibit prenylation of, or the post-prenylation reactions of, a viral protein in the manufacture of a 50 medicament for the treatment of viral infection. In preferred embodiments, the viral protein is the large delta antigen of the hepatitis D virus, core antigen of HBV, or the nef protein of HIV.

Brief Description of the Drawings

- 55 [0009]

Figures 1A and 1B are photocopies of immunoblots of proteins obtained by lysis of viral-infected cells expressing viral proteins and treated with tritiated mevalonate.

Figures 2A and 2B are photocopies of immunoblots of proteins derived from lysates of cells containing wild type or mutant viral proteins and labeled with tritiated proline or mevalonate.

Figures 3A, 3B, 3C and 3D are photocopies of immunoblots of various cell supernatants containing viral proteins.

Figure 4 is a diagrammatic representation of the progress of HDV morphogenesis.

5

Modes of Carrying Out the Invention

[0010] Hepatitis delta virus (HDV) infections cause both acute and chronic liver disease and can be fatal (1, 2). This RNA virus contains a 1.7 kb single-stranded circular genome and delta antigen, the only known HDV-encoded protein.

10 These elements are encapsulated by a lipid envelope in which hepatitis B virus surface antigens are embedded (3), which explains why HDV infections occur only in the presence of an accompanying HBV infection (4, 5). Two isoforms of delta antigen exist in infected livers and serum (6, 7). This heterogeneity arises from a unidirectional mutation at a single nucleotide in the termination codon for delta antigen (codon 196: UAG → UGG), which occurs during replication (8). Thus, although small delta antigen is 195 amino acids long, large delta antigen is identical in sequence except that 15 it contains an additional 19 amino acids at its COOH terminus. Although both forms of delta antigen contain the same RNA genome binding domain (9), they have dramatically different effects on genome replication. The small form is required for replication, whereas the large form is a potent transdominant inhibitor (10, 11).

15 [0011] The last four amino acids of large delta antigen are Cys-Arg-Pro-Gln-COOH (SEQ ID NO:2). This COOH-terminal configuration, termed a CXXX (SEQ ID NO:1) box (where C is cysteine and X is any amino acid), has been implicated as a substrate for prenyltransferases that add to the cysteine 15 (farnesyl) or 20 (geranylgeranyl) carbon moieties derived from mevalonic acid (12-14). The resulting hydrophobic modification may aid in membrane association of the derivatized protein, as suggested for p21 Ras (15, 16) and lamin B (12, 17). We have now demonstrated that large delta antigen is similarly modified.

20 [0012] Other virions also contain suitable target sequences for prenylation. These sequences are near the carboxy terminus of the viral protein targeted, and may be in the form of CXXX (SEQ ID NO:1) boxes, but the cysteine may also be closer to the C-terminus, including a position as the C-terminal amino acid, as is the case of the core antigen of hepatitis B virus (HBV) and the nef gene product of HIV-1.

25 [0013] To determine whether large delta antigen is a substrate for prenylation, we labeled three cell lines, SAG, LAG, and GP4F, with [³H]mevalonic acid. GP4F cells are a derivative of NIH 3T3 cells (18). SAG (19) and LAG (20) cells are derivatives of GP4F cells that stably express the small and large delta antigens, respectively.

30 [0014] Labeled cell lysates were analyzed on immunoblots (Fig. 1A) to detect steady-state amounts of small and large delta antigen. The lysates were also subjected to immunoprecipitation with an antibody to the delta antigens (anti-delta), SDS polyacrylamide gel electrophoresis (SDS-PAGE), and fluorography (Fig. 1B).

35 [0015] In more detail, referring to Fig. 1, large delta antigen is shown to be prenylated in cultured cells. The cell lines SAG (19) (lane 1), LAG (20) (lane 2), and GP4F (18) (lane 3) were grown overnight in Lovastatin (25 μM) and (R,S)-[5-³H]mevalonate (140 mM) (30), and lysed in RIPA buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS] (20). (A) Aliquots were subjected to immunoblot analysis (17). The blot was treated with serum from an HDV-infected patient that contained antibody to delta antigen (α-δAg) and horseradish peroxidase-conjugated rabbit antibody to human immunoglobulin G (IgG) (Promega), followed by chemiluminescence (Amersham) development. (B) Immunoprecipitates (with α-δAg) from cell extracts were subjected to SDS-PAGE and fluorography. As shown in Figure 1, S, small delta antigen, L, large delta antigen. Molecular size markers are shown at the left (in kilodaltons).

40 [0016] Thus, the large, but not the small, antigen was labeled with [³H]mevalonic acid, suggesting that large delta antigen undergoes prenylation in cultured cells.

45 [0017] We obtained similar results using *in vitro* translation reactions (13) performed in the presence of [³H]proline or [³H]mevalonate (Fig. 2). Fig. 2. also shows mutation of Cys²¹¹ of large delta antigen to Ser and loss of prenylation. *In vitro* translation reactions were performed with rabbit reticulocyte lysates (Promega) in the presence of either (A) L-[2,3,4,5-³H]proline (19 μM) (94 Ci/mmol, Amersham) or (B), [³H]mevalonate (200 μM) (30). For (A) and (B), translation reactions contained small delta antigen mRNA (lane 1); large delta antigen mRNA (lane 2); water (lane 3); or large delta antigen (Cys²¹¹ → Ser) (20) mRNA (lane 4). A portion (20 μl) of each reaction was added to 1 ml of RIPA buffer, immunoprecipitated with α-δAg, and analyzed as described (Fig. 1).

50 [0018] Both the small and the large antigens were labeled with [³H]proline (Fig. 2A), whereas only the large isoform was labeled with [³H]mevalonate (Fig. 2B). To determine whether modification by [³H]mevalonate was dependent on the presence of Cys²¹¹ in the terminal CXXX (SEQ ID NO:1) box, we constructed a mutant that contains a serine at this position (20). Cys²¹¹ is the only cysteine in large delta antigen. Mutating Cys²¹¹ to Ser did not interfere with the synthesis of large delta antigen (Fig. 2A) but abolished its modification by [³H]mevalonate (Fig. 2B).

55 [0019] The specific type of mevalonate modification of large delta antigen appears to be geranylgeranyl rather than farnesyl (21). Although the first described CXXX (SEQ ID NO:1) boxes contained aliphatic residues at the first and sec-

ond positions after Cys, other types of amino acids can be found in prenylation sites (13, 14). It is not clear whether the COOH-terminal sequence Cys-Arg-Pro-Gln-COOH (SEQ ID NO:3), which differs from that of previously described CXXX (SEQ ID NO:1) boxes, implies the existence of a novel prenylation enzyme or whether it reflects a broader substrate specificity of known prenyltransferases.

5 [0020] For HDV particle formation, delta antigen and associated genomes are presumably targeted to cell membranes that contain HBV envelope proteins. We hypothesized that prenylation of large delta antigen could be involved in this process. We first examined whether large delta antigen was sufficient for HDV-like particle formation. HBV surface antigen (HBsAg) was expressed transiently in COS-7 cells together with small or large delta antigen. Virus-like particles consisting of delta antigen packaged into HBsAg-containing envelopes were analyzed by immunoprecipitation of
10 clarified media supernatants with an antibody to HBsAg (anti-HBs).

[0021] Fig. 3 shows particle formation with large delta antigen and HBsAg. For panels (A) and (B), COS-7 cells were transiently transfected with the following plasmids: SV24H, which expresses HBV surface antigen (31), and SVLAg, which expresses small delta antigen (19) (lane 1); SV24H and SVL-large, which expresses large delta antigen (20) (lane 2); and calcium phosphate precipitate without DNA (lane 3). In (C) and (D), COS-7 cells were transfected with
15 SV24H and SVL-large (lane 4); SV24H and SVL-large (Ser²¹¹) (20) (lane 5); and calcium phosphate precipitate without DNA (lane 6). For (A) and (C), 48 hours after transfection, HBsAg-containing particles were immunoprecipitated from 2-ml aliquots of clarified media supernatants with anti-HBs (31) and subjected to immunoblot (with α - δ Ag) and chemiluminescence analyses as described (Fig. 1). For (B) and (D), the transfected cells were harvested in cell lysis buffer [50 mM Tris (pH 8.8), 2% SDS] with protease inhibitors (20), and aliquots subjected to protein immunoblot and chemiluminescence analyses. Molecular size markers are shown at the left (in kD).

[0022] The presence of delta antigen in the immunoprecipitates was assayed by immunoblot analysis (Fig. 3A). Although both small and large antigens were synthesized in the transfected cells (Fig. 3B), only the large isoform was incorporated into secreted HBsAg-containing particles (Fig. 3A). Similar selective packaging has been observed (22).

[0023] We then examined the function of mevalonate modification in this particle formation. One explanation for the
25 preferred packaging of large delta antigen is that the small antigen lacks the CXXX (SEQ ID NO:1) box and therefore cannot undergo modification. The Cys²¹¹ → Ser mutant of large delta antigen should behave like small delta antigen and not be packaged. This was indeed found to be the case. Whereas both wild-type and Ser²¹¹ mutant large antigens were synthesized in transfected cells (Fig. 3D), only the wild-type form was packaged into particles (Fig. 3C). Thus, the mutated form of large delta antigen is not prenylated and cannot form particles with HBsAg.

[0024] Our results suggest that prenylation of large delta antigen is required for the formation and release of particles containing delta antigen and HBV surface antigens. The requirement of a prenylation site for productive viral infection is further suggested by other mutations of the CXXX (SEQ ID NO:1) box (23) and by the conservation of Cys²¹¹ and a CXXX (SEQ ID NO:1) box motif among all sequenced HDV isolates (24).

[0025] The ability of large, but not small, delta antigen to be prenylated and packaged into virus particles further highlights the significance of the mutation-induced heterogeneity at the termination codon of the small delta antigen. During HDV replication, S genomes (encoding the small antigen) mutate to L genomes (encoding the large antigen). At least two effects attributable to this mutation can be distinguished (see Fig. 4). Fig. 4 shows the regulatory switch of S genomes to L genomes. During replication, S genomes encoding the small delta antigen mutate to L genomes, which encode the large delta antigen. This single base mutation has two effects on the COOH-terminus of delta antigen. The
35 first is to change the nature of the COOH-terminal amino acid; Pro (P), which enhances genome replication (20), is replaced by Gln (Q), resulting in inhibition of genome replication. The second effect is the creation of a target prenylation site (CRPQ) (SEQ ID NO:3), C, cysteine; R, arginine; P, proline; Q, glutamine.

[0026] Thus, the first effect is the conversion of an enhancer of genome replication (small delta antigen) into a potent trans-dominant inhibitor (large delta antigen) (10, 11). This dramatic difference in function appears to be determined
45 solely by the nature of the COOH-terminal amino acid with proline being sufficient to confer enhancer activity (11, 25). The second effect is the addition of a CXXX (SEQ ID NO:1) box to delta antigen, which allows the protein to be prenylated and presumably promotes its incorporation into HBsAg-containing particles. The combined effects of the switch from production of small to large delta antigen thus appear to have two roles: to suppress further genome replication and to promote the onset of packaging and virion morphogenesis.

[0027] Our results suggest prenylation as a new target for anti-HDV therapy and for antiviral therapy with respect to other viruses with prenylated proteins. Such therapy is directed at inhibiting virion morphogenesis, production, release and uncoating (functionally the reverse reaction of virion morphogenesis). In light of the increasingly apparent degeneracy of the four C-terminal amino acids required to function as a prenylation substrate, a cysteine located at any of these C-terminal positions is also considered to identify a potential target of antiprenylation therapy.

[0028] Several strategies designed to interfere with the prenylation stage of the HDV life cycle may be considered, including drugs that inhibit enzymes along the prenylation pathway, and CXXX (SEQ ID NO:1) box analogs. Both therapies have been considered for the inhibition of ras-mediated oncogenic transformation (26). Tetrapeptides that correspond to the CXXX (SEQ ID NO:1) box of p21 Ha-Ras inhibit pr nylation of p21 Ha-Ras *in vitro* (27). Finally, the dual

function of large delta antigen in the HDV life cycle suggests a further refinement of a proposed (11) defective interfering particle- (DIP) (28) like therapy aimed at cells infected with actively replicating S genomes. Because L genomes require a source of small delta antigen for replication (19, 29) but, once replicated, produce a potent trans-dominant inhibitor of further replication, a therapeutically administered L genome DIP could be specific for infected cells, as well as possess an inherent shut-off mechanism (11). If the L genome also contained the Cys²¹¹ to Ser mutation, it could encode a delta antigen that not only inhibits replication but also affects packaging.

[0029] Accordingly, new approaches to antiviral therapy and inhibition of viral morphogenesis focus on inhibition of the prenylation of, or post-prenylation reactions of, at least one viral protein. This may be effected by contacting cells infected with the target virus with an effective amount of an agent which inhibits the prenylation of, or post-prenylation reactions of, at least one viral protein. Such agents include inhibitors of formation of the prenyl groups which are derivative of the mevalonate synthesis pathway. Other agents include decoys for the target sequence for prenylation, including small peptides, including tetrapeptides and other compounds which mimic the surroundings of the cysteine residue to be prenylated. For example, Reiss, Y., et al. *Cell* (1990) 62:81-88 report prenylation inhibition by C-A-A-X tetrapeptides. As set forth above, the cysteine residue to be prenylated is generally found at the carboxy terminus of the target protein; although the most common target sequence involves a CXXX (SEQ ID NO:1) box, cysteines positioned closer to the C-terminus may also be targeted; thus, the relevant peptides may include those of the form XCXX, XXXC, and XXXC (SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6). Other agents include derivatives and mimics of prenyl groups themselves. Other suitable agents include inhibitors of the prenyltransferase enzymes and of enzymes that catalyze post-prenylation reactions.

Assay of Candidate Inhibitors

[0030] The present invention also provides a method to screen candidate drugs as prenylation inhibitors by taking advantage of the requirement for prenylation in order to effect secretion of certain prenylated proteins. For those proteins for which secretion requires prenylation, the assay can be conducted in a direct and simple manner. Cells that secrete, or that have been modified to secrete, a first protein whose secretion is dependent on prenylation are used as the experimental cells. A second protein which does not depend on prenylation for secretion is used as a control. This control protein may be secreted by the same or different host cells as the first protein. The candidate drug is applied to cells that secrete both proteins, or to matched sets of cells that secrete each. Secretion can readily be assessed by assaying the cell supernatants for the presence or absence of the first and second secreted proteins using, for example, routine ELISA assays. Successful candidate drugs will not inhibit the secretion of the control protein, but will inhibit the secretion of the protein in the test sample wherein prenylation is required for secretion.

[0031] The large delta antigen of HDV is a viral protein for which prenylation is a prerequisite for secretion. Thus, this protein forms, itself a key part of a useful test system for the assay. Cells that are modified to secrete a protein for which prenylation is not required can be used as controls. If large delta antigen is used as the test protein, it is advantageous to use HBsAg as the control protein in the same cell since HBsAg is also required for secretion of delta antigen.

[0032] The foregoing assay, of course, requires that the inhibitor interfere with the prenylation system for large delta antigen or for any other prenylation-controlled secreted protein used in the assay. A range of prenyl transferases and prenyl groups is known to apply to various proteins for which prenylation inhibitors are required or sought. Some of these proteins are not secreted, whether they are prenylated or not; one such example is the protein product of the ras oncogene.

[0033] Nevertheless, the assay system described can be employed to screen for inhibitors of prenylation in these non-secreted proteins by providing the target "CXXX" (SEQ ID NO:1) box characteristic of the nonsecreted protein in place of the corresponding "CXXX" (SEQ ID NO:1) box of the secreted one. The resulting chimeric protein will exhibit the prenylation characteristics of the imported "CXXX" (SEQ ID NO:1) box characteristic of the nonsecreted protein, but retain the ability of the host secreted protein to be passed to the supernatant for assay. Thus, the range of target proteins for which prenylation inhibitors are sought by use of the assay can be expanded to nonsecreted proteins.

[0034] The presence of a control system which provides secreted protein not dependent on prenylation is critical. The presence of this control allows candidate inhibitors which merely are toxic to the cells, or which inhibit secretion in general, to be discarded. Prenylation inhibitors identified by one of the variations of the above described assay are expected to find use not only in the inhibition of viruses, but also in other processes or disease states -- including but not limited to cancer -- in which a prenylated protein is found to be involved.

[0035] Evidently, prenylation of viral proteins is a prerequisite for additional post-prenylation reactions of the proteins such as proteolysis and carboxymethylation. The essential sequence of steps can be interfered with at the most convenient point for the viral protein in question.

Administration of the Inhibitors

[0036] Additional viral proteins subject to prenylation can be obtained by screening amino acid sequence data banks for viral proteins which contain a "CXXX" (SEQ ID NO:1) box at the C-terminus. An illustrative list of such proteins includes, for example, specific proteins of HAV, HCV, HSV, CMV, VZV, influenza virus, plant viruses such as tobacco mosaic satellite virus and barley stripe mosaic virus, core antigen of hepatitis B virus and the nef gene product of HIV I, as set forth above. These candidates for suitable prenylation targets can be validated in a manner similar to that described above by providing labeled mevalonic acid to cells infected with or containing the appropriate viruses or viral gene products, and assessing the prenylation status of the viral proteins obtained using incorporation of label as the criterion. Furthermore, the role of prenylation in the morphogenesis of the respective virions, and its suitability as a target for anti-viral therapy, can also be validated in a manner similar to that described above.

[0037] If viral morphogenesis, production, release or uncoating are to be inhibited in culture, suitable host cells are used to culture the virus, and the agents used in inhibiting prenylation or post prenylation reactions added to the medium. If the infected cells are contained in an animal subject, such as a mammalian subject or in particular a human or other primate subject, the agent used for the prenylation inhibition is generally introduced as a pharmaceutical formulation. Suitable formulations depending on the nature of the agent chosen may be found in *Remington's Pharmaceutical Sciences*, latest edition, Mack Publishing Co., Easton, PA. The routes of administration include standard such routes, including administration by injection, oral administration, and transmucosal and transdermal administration. The choice of formulation will depend on the route of administration as well as the agent chosen. Suitable mixtures of agents can also be used as active ingredients. For administration to plants, formulations which are capable of conducting the active ingredients into plant cells are used as carriers.

[0038] The following references are listed according to the number which refers to them in the body of the specification:

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the protein from an inhibitor to an enhancer of genome replication (20).

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10 32. We thank J.-J. Gonvers for providing human anti-delta antigen serum, A. Alberts for providing Lovastatin, and J.M. bishop, H. Bourne, and D. Ganem for helpful discussions and critical reading of the manuscript. J.M.W. is a recipient of an NIH grant and J.S.G. was supported by the Medical Scientist Training Program.

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT: GLENN, JEFFREY

(ii) TITLE OF INVENTION: METHOD FOR INHIBITION OF VIRAL
MORPHOGENESIS

10 (iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: FISHER & AMZEL
- (B) STREET: 1320 HARBOR BAY PARKWAY, STE 225
- (C) CITY: ALAMEDA
- (D) STATE: California
- (E) COUNTRY: USA
- (F) ZIP: 94501

15 (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

20 (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US 07/890,754
- (B) FILING DATE: 29-MAY-1992
- (C) CLASSIFICATION:

25 (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: FISHER, STANLEY P.
- (B) REGISTRATION NUMBER: 24,344
- (C) REFERENCE/DOCKET NUMBER: 92-164-2PCT

30 (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (510) 748-6868
- (B) TELEFAX: (510) 748-6688

35 (2) INFORMATION FOR SEQ ID NO:1:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Xaa Xaa Xaa
1

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(2) INFORMATION FOR SEQ ID NO:2:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Arg Pro Gln
1

15 (2) INFORMATION FOR SEQ ID NO:3:

- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 20 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "This position is Gln-COOH."

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Arg Pro Gln
1

30 (2) INFORMATION FOR SEQ ID NO:4:

- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Xaa Cys Xaa Xaa
1

45 (2) INFORMATION FOR SEQ ID NO:5:

- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Xaa Cys Xaa
1

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Xaa Xaa Cys
1

Claims

- 20 1. The use of an agent which inhibits the prenylation of, or a post-prenylation reaction of, at least one viral protein in
the manufacture of a medicament for the inhibition of virion morphogenesis, production, release or uncoating.

25 2. The use according to claim 1, wherein the agent is an inhibitor of prenyl group synthesis, is an inhibitor of prenyl
transferase, the agent mimics a prenyl group or mimics the prenylation locus of the viral protein.

30 3. The use according to claim 1 or 2, wherein the viral protein contains a C-terminal amino acid sequence of the for-
mula CXXX, XCXX, XXCX or XXXC wherein C is cysteine and each X independently presents any amino acid
and/or the agent interferes with a post-prenylation reaction.

35 4. The use according to any preceding claim wherein the virion is hepatitis D virus (HDV) and the viral protein is the
large delta antigen of HDV, and preferably the inhibition is effected by a transdominant inhibitor of replication mod-
ified to resist prenylation.

40 5. The use according to any one of claims 1 to 3 wherein the virion is human immunodeficiency virus and the viral
protein is the nef protein.

6. The use of an agent which inhibits the prenylation of, or inhibits a post-prenylation reaction of, a viral protein in the
manufacture of a medicament for the treatment of viral infection.

45 7. A method of inhibiting virion morphogenesis, production, release or uncoating in a plant, the method comprising
administering to the plant an agent which inhibits the prenylation of, or inhibits a post-prenylation reaction of, a viral
protein.

8. A method of screening a candidate drug as a prenylation inhibitor, the method comprising:

50 (a) providing cells which secrete or which have been modified to secrete a first protein containing a "CXXX"
box and second control protein where secretion of the first protein is dependent on prenylation and secretion
of the second control protein is not dependent on prenylation, wherein the cells that secrete the first and sec-
ond proteins respectively may be the same or different;

55 (b) contacting the cells with a candidate drug under conditions wherein the control second protein is secreted;
and

(c) determining the presence, absence or amount of the first protein secreted from the cells;
wherein a candidate drug which decreases or abolishes the amount of secreted first protein is an effective pre-
netylation inhibitor.

9. A method according to claim 8 wherein the first protein is a large delta antigen.

10. A method according to claim 8 wherein the first protein is a chimera consisting of a natively secreted protein which has been modified to contain, in place of its "CXXX" box, the "CXXX" box of a different protein.

Patentansprüche

- 5 1. Verwendung eines Mittels, welches die Prenylierung oder eine Nach-Prenylierungsreaktion mindestens eines viralen Proteins inhibiert, bei der Herstellung eines Medikamentes zur Inhibierung der Virion-Morphogenese, -Produktion, -Freisetzung oder -Enthüllung bzw. -Hüllöffnung.
- 10 2. Verwendung gemäß Anspruch 1, wobei das Mittel ein Inhibitor der Prenylgruppen-Synthese ist, ein Inhibitor von Prenyltransferase ist, das Mittel eine Prenylgruppe nachahmt oder die Prenylierungsstelle des viralen Proteins nachahmt.
- 15 3. Verwendung gemäß Anspruch 1 oder 2, wobei das virale Protein eine C-terminale Aminosäuresequenz der Formel CXXX, XCXX, XXCX oder XXXC enthält, worin C Cystein ist und jedes X unabhängig für eine Aminosäure steht, und/oder das Mittel eine Nach-Prenylierungsreaktion stört.
- 20 4. Verwendung gemäß einem der vorhergehenden Ansprüche, worin das Virion Hepatitis-D-Virus (HDV) ist und das virale Protein das große Delta-Antigen von HDV ist und die Inhibierung vorzugsweise durch einen transdominannten Inhibitor der Replikation bewirkt wird, entsprechend modifiziert zur Resistenz gegen Prenylierung.
- 25 5. Verwendung gemäß mindestens einem der Ansprüche 1 bis 3, worin das Virion ein humaner Immunodefekt-Virus ist und das virale Protein das nef-Protein ist.
- 30 6. Verwendung eines Mittels, welches die Prenylierung oder eine Nach-Prenylierungsreaktion eines viralen Proteins inhibiert, bei der Herstellung eines Medikamentes zur Behandlung viraler Infektion.
- 35 7. Verfahren zur Inhibierung der Virion-Morphogenese, -Freisetzung und -Enthüllung in einer Pflanze, wobei das Verfahren das Verabreichen eines Mittels an die Pflanze umfaßt welches die Prenylierung oder eine Nach-Prenylierungsreaktion eines viralen Proteins inhibiert;
- 40 8. Verfahren des Screenens eines Kandidaten-Arzneistoffes als einem Prenylierungsinhibitor, wobei das Verfahren folgendes umfaßt:
- 35 (a) Bereitstellen von Zellen, welche ein erstes Protein, das eine "CXXX"-Box enthält, und ein zweites Kontrollprotein sezernieren oder zur Sezernierung modifiziert wurden, wobei die Sezernierung des ersten Proteins von der Prenylierung abhängt und die Sezernierung des zweiten Kontrollproteins von der Prenylierung nicht abhängt, wobei die Zellen, welche die ersten bzw. zweiten Proteine sezernieren, gleich oder unterschiedlich sein können;
- 40 (b) Kontaktieren der Zellen mit einem Kandidaten-Arzneistoff unter Bedingungen, unter denen das zweite Kontrollprotein sezerniert wird; und
- 45 (c) Bestimmen der Anwesenheit, Abwesenheit oder der Menge des ersten von den Zellen sezernierten Proteins;
- 45 wobei ein Kandidaten-Arzneistoff, welcher die Menge des sezernierten ersten Proteins vermindert oder aufhebt, ein wirksamer Prenylierungsinhibitor ist.
- 50 9. Verfahren gemäß Anspruch 8, wobei das erste Protein ein großes Delta-Antigen ist.
- 50 10. Verfahren gemäß Anspruch 8, wobei das erste Protein ein Chimeres ist, bestehend aus einem nativ sezernierten Protein, welches dahingehend modifiziert worden ist, daß es anstelle seiner "CXXX"-Box die "CXXX"-Box eines anderen Proteins enthält.

Revendications

- 55 1. Utilisation d'un agent qui inhibe la prénylation ou une réaction de postprénylation d'au moins une protéine virale dans la préparation d'un médicament pour l'inhibition de la morphogénèse, de la production, de la libération, ou de la mise à nu d'un virion.

2. Utilisation selon la revendication 1, dans laquelle l'agent est un inhibiteur de la synthèse du groupe prényl, un inhibiteur de la prényl transférase, l'agent mime un groupe prényl ou mime le site de prénylation de la protéine virale.
3. Utilisation selon la revendication 1 ou 2, dans laquelle la protéine virale contient une séquence d'acide aminé en C-terminale de formule CXXX, XCXX, XXXX ou XXXC dans laquelle C est une cystéine , et chaque X représente indépendamment toute acide aminé et/ou l'agent qui interfère avec une réaction de post-prénylation.
4. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle le virion est le virus de l'hépatite D (HDV) et la protéine virale est le grand antigène delta de HDV, et de préférence l'inhibition est effectuée par un inhibiteur transdominant de la réPLICATION qui est modifié pour résister à la prénylation.
5. Utilisation selon l'une quelconque des revendications 1 à 3 dans laquelle le virion est un virus immunodéficient et la protéine virale est la protéine nef.
6. Utilisation d'un agent qui inhibe la prénylation, ou inhibe une réaction de post-prénylation d'une protéine virale dans la préparation d'un médicament pour le traitement d'une l'infection virale.
7. Une méthode d'inhibition de la morphogénèse, de la production, de la libération ou de la mise à nu d'un virion dans une plante, ladite méthode comprenant l'administration à la plante d'un agent qui inhibe la prénylation, ou inhibe une réaction de post-prénylation d'une protéine virale.
8. Une méthode de criblage d'un médicament candidat en tant qu'inhibiteur de la prénylation, ladite méthode comprenant :
 - a) l'obtention de cellules qui secrètent ou qui ont été modifiées pour sécréter une première protéine contenant une boîte "CXXX" et une deuxième protéine contrôle, la sécrétion de la première protéine est dépendante de la prénylation et la sécrétion de la seconde protéine contrôle n'est pas dépendante de la prénylation, dans laquelle les cellules qui secrètent respectivement la première et la seconde protéine peut être la même ou différente ;
 - b) la mise en contact des cellules avec un médicament candidat dans des conditions où la seconde protéine contrôle est secrétée ;
 - c) la détermination de la présence, de l'absence, ou de la quantité de la première protéine secrétée par les cellules ;
 dans laquelle le médicament candidat qui diminue ou abolit la quantité de la première protéine secrétée est un inhibiteur efficace de la prénylation.
9. Une méthode selon la revendication 8 dans laquelle la première protéine est le grand antigène delta.
10. Une méthode selon la revendication 8, dans laquelle la première protéine est une chimère consistant en une protéine naturellement secrétée qui a été modifiée afin de contenir à la place de la boîte "CXXX", la boîte "CXXX" d'une protéine différente.

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FIG._1A

FIG._1B



1 2 3 4

FIG._2A

1 2 3 4

FIG._2B

